

IN THE SPECIFICATION:

The paragraph beginning at page 28, line 22 has been amended as follows:

In 100 ml of a tris-hydrochloric acid-calcium chloride buffer (TRIZMA BASE 0.05 mol/l, calcium chloride 0.01 mol/l, pH 7.5) was dissolved 2.6 g of an egg-derived crude SGP (sialyl glycopeptide). 58 mg (772 μ mol) of sodium azide and 526 mg of Actinase-E (manufactured by Kaken Pharmaceutical Co., Ltd.) were added to this solution, and the mixture was allowed to stand at 37 °C. After 65 hours, 263 mg of Actinase-E was added again, and the mixture was allowed to stand at 37 °C. for additional 24 hours. This solution was freeze dried, and thereafter the residue was purified twice by gel filtration column chromatography (~~Sephadex~~ SEPHADEX G-25, 2.5 ϕ × 1 m, eluent: water, flow rate: 1.0 ml/min), to give 1.3 g (555 μ mol) of a desired α 2,6-asparagine-linked disialooligosaccharide.

The paragraph beginning at page 30, line 4 has been amended as follows:

α 2,6-Asparagine-linked disialooligosaccharide (609 mg, 261 μ mol) obtained in Reference Example 1 was dissolved in 20.7 ml of water, and 13.8 ml of 0.1 N hydrochloric acid was added thereto. Immediately after heating this solution at 70 °C for 35 minutes,

the solution was cooled on ice, and a saturated aqueous sodium hydrogencarbonate was added thereto to adjust its pH 7. The solution was freeze dried, and thereafter the residue was purified by gel filtration column chromatography (~~Sephadex~~ SEPHADEX G-25, 2.5 ϕ × 1 m, eluent: water, flow rate: 1.0 ml/min), to give 534 mg of a mixture of α 2,6-asparagine-linked disialooligosaccharide, two kinds of α 2,6-asparagine-linked monosialooligosaccharide and asparagine-linked asialooligosaccharide. These four components were proceeded to the next step without being isolated from each other.

The paragraph beginning at page 30, line 26 has been amended as follows:

A 429-mg quantity of the obtained oligosaccharides mixture was dissolved in 16.3 ml of acetone and 11.2 ml of water. To the solution were added 9-fluorenyl methyl-N-succinimidyl carbonate (155.7 mg, 461.7 μ mol) and sodium hydrogencarbonate (80.4 mg, 957 μ mol), and the mixture was stirred at room temperature for 2 hours. This solution was applied to an evaporator to remove acetone, and the remaining solution was purified by gel filtration column chromatography (~~Sephadex~~ SEPHADEX G-25, 2.5 ϕ × 1 m, eluent: water, flow rate: 1.0 ml/min), to give 309 mg of a mixture of Compound 1, ~~Compounds~~ Compounds 2 and 3, and Compound 4. This mixture was

purified by HPLC (ODS column, eluent: 50 mM aqueous ammonium acetate:methanol=65:35, 2.0 ϕ × 25 cm, flow rate: 3 ml/min). As a result, Compound 1 was eluted after 51 minutes, a mixture of Compounds 2 and 3 was eluted after 67 minutes, and Compound 4 was eluted after 93 minutes. Each of the fractions were collected and freeze dried, and thereafter desalted by gel filtration column chromatography (~~Sephadex~~ SEPHADEX G-25, 2.5 ϕ × 30 cm, eluent: water, flow rate: 1.0 ml/min), thereby giving 150 mg of a desired mixture of Compounds 2 and 3.

The paragraph beginning at page 33, line 13 has been amended as follows:

The mixture of Compounds 2 and 3 obtained in Reference Example 2 (5.0 mg, 2.2 μ mol) were dissolved in 220 μ L of water, and 100 μ L of a 22 mM aqueous cesium carbonate was added thereto to adjust its pH 7.0. This solution was freeze dried. Four-hundred and thirty microliters of N,N-dimethylformamide was added to the solid obtained after drying, and further 20 μ L of a 6.6 μ mol benzyl bromide/N,N-dimethylformamide solution was added thereto. This solution was stirred under argon atmosphere. After 48 hours, the disappearance of the starting material was confirmed by TLC

(eluent: 1M NH₄OAc:isopropanol=1:2), and thereafter 4.4 mL of diethyl ether was added to the solution to allow the compound to precipitate therefrom. The precipitated oligosaccharides were filtered, and the residual oligosaccharide was dissolved in water and freeze dried. The residue after the lyophilization was purified by fractional HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate : acetonitrile=78:22, flow rate: 4 mL/min), and Compound 3 was eluted after 88 minutes and Compound 2 was eluted after 91 minutes. The fractions were collected, and further desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a benzyl derivative of Compound 2 in an amount of 1.6 mg and a benzyl derivative of Compound 3 in an amount of 1.8 mg.

The paragraph beginning at page 35, line 12 has been amended as follows:

A benzyl compound of Compound 3 (decasaccharide, 5.0 mg, 2.1 mmoles) was dissolved in 2.0 ml of NaOH aq. (pH=12) with ice cooling. The solution was stirred for about 5 hours while monitoring the reaction by HPLC. On completion of progress of the reaction, the reaction mixture was adjusted to [[PH]] pH 7.0 with

40 mM of HCl. The neutralized mixture was filtered with a membrane filter, followed by concentration, and fractionation and purification by JPLC (YMC- pack ODS-AM, SH-343-5AM, 20 × 250 mm, AN/25 mM AcONH₄ buffer=20/80, 7.0 ml/min., wave length: 274 nm). The fraction obtained was concentrated and passed through an ODS column (~~Cosmosil~~ COSMOSIL 75C₁₈-OPN, product of NACALAI TESQUE, INC.) for a desalting treatment, followed by concentration and freeze-drying to obtain the desired product, i.e., Compound 3 (2.5 mg, 52.0%) The compound obtained had the following physical data. Table 1 shows the structure of Compound 3 as simplified.

The paragraph beginning at page 40, line 25 has been amended as follows:

Compound 5 (28 mg, 21.3 μmol) and 1.0 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 454 μL), and neuraminidase (manufactured by Sigma-Aldrich Corporation, from Vibrio Cholerae, 198 mU) was added thereto. This solution was allowed to stand at 37 °C for 20 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. The reaction solution was purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further,

the residue was desalted on ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 11 (17 mg, yield: 70%). The physical data for the resulting compound are as follows. Table 2 shows the structure of Compound 11 as simplified.

The paragraph beginning at page 41, line 25 has been amended as follows:

Compound 6 (20 mg, 9.4 μmol) and 1.6 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 323 μL), and neuraminidase (manufactured by Sigma-Aldrich Corp., from Vibrio Cholerae, 141 mU) was added thereto. This solution was allowed to stand at 37 °C for 18 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. Subsequently, the reaction solution was purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further, the residue was desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 12 (13 mg, yield: 76%). The structure of the resulting compound was confirmed from the

finding that its ¹H-NMR was identical to that of the standard compound. Table 2 shows the structure of Compound 12 as simplified.

The paragraph beginning at page 42, line 15 has been amended as follows:

Compound 7 (45 mg, 24 μmol) and 1.7 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 820 μL), and neuraminidase (manufactured by Sigma-Aldrich Corp., from Vibrio Cholerae, 134 mU) was added thereto. This solution was allowed to stand at 37 °C for 14 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. Subsequently, the reaction solution was purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further, the residue was desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 13 (28 mg, yield: 74%). The physical data for the resulting compound are as follows. Table 2 shows the structure of Compound 13 as simplified.

The paragraph beginning at page 43, line 14 has been amended as follows:

Compound 8 (47 mg, 25 μ mol) and 1.9 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 840 μ L), and neuraminidase (manufactured by Sigma-Aldrich Corp., from Vibrio Cholerae, 369 mU) was added thereto. This solution was allowed to stand at 37 °C for 37 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. The reaction solution was freeze dried, and the freeze dried product was subsequently purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further, the residue was desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 14 (26 mg, yield: 65%). The physical data for the resulting compound are as follows. Table 2 shows the structure of Compound 14 as simplified.

The paragraph beginning at page 44, line 13 has been amended as follows:

Compound 9 (32 mg, 18.4 μ mol) and 2.5 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 713 μ L), and neuraminidase (manufactured by Sigma-Aldrich Corp., from Vibrio Cholerae, 134 mU) was added thereto. This solution was allowed to

stand at 37 °C for 17 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. Subsequently, the reaction solution was purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further, the residue was desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 15 (13 mg, yield: 52%). The physical data for the resulting compound are as follows. Table 2 shows the structure of Compound 15 as simplified.

The paragraph beginning at page 45, line 11 has been amended as follows:

Compound 10 (28 mg, 16 μmol) and 1.7 mg of bovine serum albumin were dissolved in HEPES buffer (50 mM, pH 5.0, 624 μL), and neuraminidase (manufactured by Sigma-Aldrich Corp., from Vibrio Cholerae, 117 mU) was added thereto. This solution was allowed to stand at 37 °C for 17 hours, and thereafter the termination of the reaction was confirmed by HPLC analysis. Subsequently, the reaction solution was purified by HPLC (YMC Packed Column D-ODS-5 S-5 120A ODS No. 2020178, 20 × 250 mm, eluent: 50 mM aqueous ammonium acetate:acetonitrile=80:20, flow rate: 4 mL/min). Further, the

residue was desalted on an ODS column (~~Cosmosil~~ COSMOSIL 75C18-OPN, 15 × 100 mm, eluted first with 50 mL of H₂O and then with 25% acetonitrile), to give a desired Compound 16 (14.6 mg, yield: 68%). The physical data for the resulting compound are as follows. Table 2 shows the structure of Compound 16 as simplified.

The paragraph beginning at page 49, line 12 has been amended as follows:

¹H-NMR and ¹³C-NMR were measured using Bruker's AVANCE 400 (mentioned as 400 MHz). When the solvent was deuteriochloroform, trimethylsilane was used as internal standard. When other deuteriated solvents were used, the peak of the solvent was used as a reference. Chemical shifts were indicated by δ (ppm), and the coupling constants by J (Hz). Used for silica gel chromatography were Merck Silicagel 60, 70-230 mesh or 230-400 mesh, and spherical silica gel which was Silica Gel 60 (Spherical), product of Kanto Chemical Co., Ltd. Used for detecting reactions (for TLC) was DC-Platten Kieselgel 60 F254 (Artl, 05715), product of E. Merk. The columns used for high performance chromatography (HPLC) were ~~Cosmosil~~ COSMOSIL 5C₁₈-AR Packed Column [φ4.6 ×150 mm], product of NACALAI TESQUE, INC.. The spectrophotofluorometer used was FP-210 Spectrofluorometer, product of JASCO.

The paragraph beginning at page 55, line 3 has been amended as follows:

Compound 24 (50 mg, 0.224 mmol), sodium piruvate (123 mg, 1.12 mmols) and bovine serum albumin (5 mg) were dissolved in a sodium phosphate buffer solution (100mM, pH 7.5, 3.4 ml), and aldolase sialate was thereafter added to the solution to start a reaction at room temperature. The reaction mixture was freeze-dried 24 hours later. The product was dissolved in a small amount of water and applied to an anion-exchange resin column (AG 1-X8, 200-400 mesh, formate form). After passing 300 ml of water through the column, the desired product was eluted with 1M formic acid, and the eluate was concentrated under the decompression. The residue was purified with a gel filtration column (~~Sephadex~~ SEPHADEX G-15, water), giving Compound 25 (40 mg, yield 58.9%).

The paragraph beginning at page 63, line 5 has been amended as follows:

Compound (C1-2) (2 mg, 0.88 μ mole) obtained in Example 1 and 1 mg of bovine serum albumin were dissolved in 100 μ l of HEPES buffer solution (50 mM, pH 5.0), and β -galactosidase (product of Seikagaku Corp., from Jack Beans, 5 μ l, 100 mU) was added to the solution. The resulting solution was allowed to stand at 37°C for

15 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) × 25 cm; eluent: 50 mM aqueous solution of ammonium acetate : acetonitrile = 82:18; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was dissolved in 200 µl of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.5 µg of the desired Compound (C2). The NMR data is given below.

The paragraph beginning at page 64, line 10 has been amended as follows:

Compound (C2) (1.8 mg, 0.86 µmole) obtained in Example 2 and 1 mg of bovine serum albumin were dissolved in 90 µl of HEPES buffer solution (50 mM, pH 5.0), and 4 µl (250 mU) of N-acetyl-β-glucosamidase (product of Sigma-Aldrich Corp., from Jack Beans) was added to the solution. The resulting solution was allowed to stand at 37°C for 24 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) × 25 cm; eluent: 50 mM aqueous solution of ammonium acetate : acetonitrile = 82:18; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was

dissolved in 200 μ l of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.9 μ g of the desired Compound (C3).

The paragraph beginning at page 65, line 9 has been amended as follows:

Compound (C3) (0.8 mg, 0.42 μ mole) obtained in Example 3 and 1 mg of bovine serum albumin were dissolved in 50 μ l of HEPES buffer solution (50 mM, pH 5.0), and 30 μ l (2.9 U) of α -mannosidase (product of Sigma-Aldrich Corp., from Jack Beans) was added to the solution. The resulting solution was allowed to stand at 37°C for 63 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) \times 25 cm; eluent: 50 mM aqueous solution of ammonium acetate : acetonitrile = 80:20; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was dissolved in 200 μ l of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.6 μ g of the desired Compound (C4).

The paragraph beginning at page 66, line 10 has been amended as follows:

Compound (C1-3) (1 mg, 0.44 μ mole) obtained in Example 1 and 1 mg of bovine serum albumin were dissolved in 50 μ l of HEPES buffer solution (50 mM, pH 5.0), and β -galactosidase (product of Seikagaku Corp., from Jack Beans, 5 μ l, 100 mU) was added to the solution. The resulting solution was allowed to stand at 37°C for 15 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) \times 25 cm; eluent: 50 mM aqueous solution of ammonium acetate : acetonitrile = 82:18; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was dissolved in 200 μ l of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.3 μ g of the desired Compound (C5).

The paragraph beginning at page 67, line 9 has been amended as follows:

Compound (C5) (1.0 mg, 0.48 μ mole) obtained in Example 5 and 1 mg of bovine serum albumin were dissolved in 50 μ l of HEPES buffer solution (50 mM, pH 5.0), and 4 μ l (250 mU) of N-acetyl- β -

glucosamidase (product of Sigma-Aldrich Corp., from Jack Beans) was added to the solution. The resulting solution was allowed to stand at 37°C for 22 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) × 25 cm; eluent: 50 mM aqueous solution of ammonium acetate: acetonitrile = 82:18; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was dissolved in 200 µl of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.6 µg of the desired Compound (C6).

The paragraph beginning at page 68, line 10 has been amended as follows:

Compound (C6) (1.0 mg, 0.53 µmole) obtained in Example 6 and 1 mg of bovine serum albumin were dissolved in 50 µl of HEPES buffer solution (50 mM, pH 5.0), and 10 µl (0.9 U) of α-mannosidase (product of Sigma-Aldrich Corp., from Jack Beans) was added to the solution. The resulting solution was allowed to stand at 37°C for 20 hours, and thereafter filtered with a membrane filter. The filtrate was purified by HPLC [ODS column, 2.0 (diam.) × 25 cm; eluent: 50 mM aqueous solution of ammonium acetate: acetonitrile =

80:20; flow rate 7.5 ml/min], followed by concentration of the solvent and freeze-drying. The residue was dissolved in 200 µl of water and desalted by ODS-column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-opn, washing with water first, subsequent elution with 25% aqueous solution of acetonitrile), giving 0.5 µg of the desired Compound (C7).

The paragraph beginning at page 89, line 12 has been amended as follows:

All of the asparagine-linked oligosaccharide derivatives were subjected to the deprotection of the Fmoc group in accordance with the following procedures. First, 240 µL of N,N-dimethylformamide and 160 µL of morpholine were added per 1 µmol of the Fmoc form of the sugar chain asparagine, and the resulting mixture was subjected to reaction at room temperature under argon atmosphere. The termination of the reaction was confirmed by TLC (eluent: 1M ammonium acetate:isopropanol=8:5), and thereafter the mixture was cooled with ice water. To this mixture was added diethyl ether in an amount of 10 times that of the reaction solution, with stirring the mixture for 15 minutes, and thereafter the precipitates formed were filtered. The residue obtained was dissolved in water, and

evaporated at 35 °C. Further, a procedure of adding 3 mL of toluene thereto and evaporating the mixture was repeated three times. The residue was purified by reverse phase column chromatography (~~Cosmosil~~ COSMOSIL 75C₁₈-OPN, 15 × 100 mm, eluent: water) to obtain corresponding asparagine-linked oligosaccharides.